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# **Genetic diversity studies of grasscutter (*Thryonomys swinderianus*) in Ghana by microsatellite and mitochondrial markers**

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### **Chapter 1. General introduction**

Grasscutter is a fairly large rodent that inhabits sub-saharan Africa, mainly Western, Central, Southern and some parts of East Africa. The species is hunted aggressively for its meat in West Africa because of the people's fondness for the meat. There have been efforts over the years to domesticate this species to augment the protein needs of the people in West Africa and subsequently in other parts of the continent depending on acceptability. Many hunting techniques including the use of fire are employed to hunt the species. The use of fire most often gets out of hand leading to bushfires that destroy the habitats of many wildlife species, thereby raising serious biodiversity and environmental issues. Even though there are concerns about the techniques used for hunting the grasscutter in the wild, the grasscutter bushmeat trade continues to flourish, making significant contributions to the Ghanaian economy as well as the economies of other African countries where the bushmeat is consumed. Efforts are ongoing to fully domesticate the grasscutter in order to increase protein supply in the sub-region and to forestall the negative consequences of hunting the species. Genetic improvement is therefore needed to enhance the domestication process but genetic information available is very limited. The aim of this study was to develop microsatellite markers that would complement the genetic improvement efforts and to determine the genetic diversity of grasscutter populations in order to identify unique populations and genotypes which would be used to set up a reference family. This study is organised into two parts. In the first part (Chapter 2), I developed novel microsatellite markers in the grasscutter for the first time and in the second part (Chapter 3) I determined the genetic diversity and structure of the grasscutter populations in Ghana using non-coding maternally inherited marker (mitochondrial D-loop) and neutral bi-parentally inherited autosomal microsatellite markers selected from the first part of this study (Chapter 2) based on their level of polymorphism. Increasing our understanding of the genetic structure, population diversity, dispersal and other population level mechanisms will inform conservation management decisions in the future if the need arises.

## **Chapter 2. Development of microsatellite markers for grasscutter (*Thryonomys swinderianus*, RODENTIA) using next-generation sequencing technology**

Microsatellite markers or short tandem repeats (STR) are DNA sequences consisting of tandem repeats of up to six nucleotides which are distributed ubiquitously in the genome of prokaryotes and eukaryotes. Among their many uses include genotyping and identification of individuals, parentage analysis and inference of kin relationships, mapping of genomes and population genetics studies. The aim of this study was to develop as many microsatellite markers as possible which will later be used to map the genome of the grasscutter.

Genomic DNA extracted from a male grasscutter was sequenced using the next generation sequencer which generated a total of 704,466 sequence reads with the average read length of 377 bp. The data were screened for microsatellite repeats which resulted in the development of 7,702 primer pairs out of which 404 were tested and 116 were found to be polymorphic. Each marker was screened using a three step approach; initial amplification was carried out using four individuals (two males and two females) followed by genotyping of a family of six individuals (sire, dam and four offspring) to check Mendelian inheritance and finally genotyping of 16 unrelated individuals. Successful amplification of all markers in both sexes suggested that none of the markers was Y chromosome-linked (Y-STR). The number of alleles per locus ranged from 2 to 13 (mean 6.5) while the observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosities ranged from 0.063 to 1.000 (mean 0.575) and 0.063 to 0.919 (mean 0.723), respectively. Cumulative probability of identity (PI) for all loci was very low ( $PI = 6.5 \times 10^{-111}$  for unrelated individuals and  $2.3 \times 10^{-42}$  when siblings are involved), indicating the usefulness of these markers for individual discrimination.

## **Chapter 3. Genetic diversity of grasscutter in Ghana**

### **3.1 Mitochondrial D-loop Diversity of Grasscutter (*Thryonomys swinderianus*, RODENTIA, HYSTRICOMORPHA) in Ghana**

Grasscutters are widely distributed in Ghana but are largely not studied, making population level information lacking. The objective of this study was to determine genetic diversity of grasscutter populations in Ghana using mitochondrial marker (D-loop). DNA was extracted from roots of hair samples collected from 84 grasscutters from three agro-ecological zones in Ghana, namely Guinea Savanna ( $n = 17$ ), Forest ( $n = 22$ ), and Coastal Savanna ( $n = 45$ ). Mitochondrial D-loop was sequenced and the diversity determined across zones. A total of 26 haplotypes were found across all zones. Out of the total number of haplotypes, 15 were obtained from Guinea Savanna, seven from Forest and 13 from Coastal Savanna. Two haplotypes were found to be common to all zones indicating gene flow among the different populations. Haplotype diversities were 0.978, 0.853 and 0.875 respectively for Guinea Savanna, Forest and Coastal Savanna zones. Analysis of molecular variance (AMOVA) revealed significant differentiation between Forest and Savanna populations ( $F_{ST} = 0.14$ ,  $p < 0.05$ ). Network analysis indicated two clusters, one of which consisted of only Savanna haplotypes and the other cluster consisted of haplotypes from both Savanna and Forest. Population neutrality tests showed that Forest and Coastal Savanna populations have been stable while the Guinea Savanna zone population had undergone an expansion in the past (Fu's  $F_S = -7.132$ ,  $p < 0.05$ ). The results of this study demonstrated that the Ghanaian populations of grasscutters are highly diverse but are less distinctive.

### 3.2 Genetic diversity of grasscutter (*Thryonomys swinderianus*) in Ghana based on microsatellite markers

The objective of this study was to apply the novel microsatellite markers developed in the previous study to determine the structure and diversity of grasscutter populations in Ghana. A total of 66 hair samples were collected from grasscutters hunted from the wild by hunters and farmers in three agro-ecological zones of Ghana. In Guinea Savanna, samples were taken from around the Mole national park ( $n = 10$ ) and Tamale ( $n = 9$ ). Sixteen samples were collected from a bushmeat market in Kumasi which is located in the Forest zone. In the Coastal Savanna zone, samples were taken from Mankessim ( $n = 5$ ), Jukwa ( $n = 7$ ) and Accra ( $n = 4$ ). Additionally, eight samples were taken from Nkwanta and seven from Afajato area, both located in the Volta Region which is at the eastern side of the Volta Lake. This was done in order to test the effect of the Volta Lake on the grasscutter populations in the Guinea Savanna zone. DNA was extracted from 1-2 mm hair root clippings of 15-20 hair pieces and samples were genotyped using 12 highly polymorphic microsatellite markers. The results show that within population level diversity was quite high ( $MN_A = 7.3$ ,  $H_E = 0.745$ ), indicating high level of variation within populations.  $F_{IS}$  which shows the level of inbreeding was found to be low ( $F_{IS} = 0.015$ ), indicating that the populations are not inbred. Phylogenetic analysis revealed that Forest population is closer to the Coastal Savanna population than other populations whilst Volta Region population is closer to the Guinea Savanna population than other populations. Pairwise  $F_{ST}$  values however indicated that all populations were significantly differentiated ( $p < 0.01$ ). STRUCTURE clustering analysis showed that Volta population split from the Guinea Savanna population which could be due to the Volta River which might serve as a barrier to gene flow.

### Chapter 4. General discussion and final remarks

Several microsatellite markers have been developed for the first time in grasscutter through this study. The markers will serve as very useful resources for mapping the genome of the grasscutter and also serve as effective tools to understand the phylogeography, dispersal patterns and genetic structure of wild grasscutter populations. Mapping the grasscutter genome will enable us to identify regions in the genome that are responsible for certain traits and ultimately find the genes that underlie such phenotypes. This approach will tremendously enhance the grasscutter domestication in order to improve protein supply in Sub-Saharan Africa. Genome research on the grasscutter will benefit immensely from model species such as rat and mouse which have been studied extensively.

In this study, it has been revealed that a lot of genetic variation exists within grasscutter populations in Ghana which shows genetic potential which could be exploited for genetic improvement. There remains a lot to be known about the grasscutter ecology. Even though the grasscutter is not an endangered species, understanding of the population dynamics may help to monitor them for future conservation. Population level studies across the species range may identify unique populations that will inform conservation decisions in the future if the need arises. Furthermore, microsatellite analysis of groups in the wild will help us to understand the social structure of the grasscutter which will be useful for the management of the grasscutters kept under domestic conditions.